BIO-FUNCTIONAL MAGNETIC NANOPARTICLES FOR MR MONITORING AND LOCALIZED HYPERTHERMIC TREATMENT OF CANCER

P. G. Kyrtatos*[1,2], M. R. Loebinger*[3], A. N. Price[1], M. Kallumadil[4,5], P. Southern[4,5], Q. A. Pankhurst[4,5], S. M. Janes[3], and M. F. Lythgoe[1,2]

[1]Centre for Advanced Biomedical Imaging, UCL Institute of Child Health and UCL Department of Medicine, London, United Kingdom, [2]RCS Unit of Biophysics, UCL Institute of Child Health, London, United Kingdom, [3]Centre for Respiratory Research, University College London, [4]London Centre for Nanotechnology, [5]Davy-Faraday Research Laboratories, The Royal Institution of Great Britain, London, United Kingdom, *equal contribution to this work

Introduction: Lung cancer is the biggest cancer killer (1). Recent evidence suggests that stromal tissue within cancers can be mesenchymal stem cell (MSC) derived (2,3). Superparamagnetic iron oxide nanoparticles (SPIO) offer attractive possibilities in biomedicine as they can be utilized for MR imaging and targeted localized hyperthermia by application of RF magnetic fields (4,5). The long term aim of our study is to utilize the tumor-homing capacity of MSCs to deliver a payload of nanoparticles for targeted hyperthermia and non-invasive MR monitoring of cancer therapy. Here we present preliminary data on particle heating using a custom-made RF applicator, MSC labeling and tumor imaging.

Methods

Hyperthermia. Several SPIO particles were previously screened for their ability to produce heat when placed in an alternating magnetic field (5). In this study we used Chemicell NC-D 200nm 2mg/mL. A 12 kA/m, 1.05 MHz field was applied for 20 min with continuous temperature monitoring.

MSCs and SPIO labeling. MSCs were obtained from Tulane University, New Orleans, USA, cultured in αMEM/16% FBS and fluorescently labeled with DiI. For SPIO labeling, cells were cultured with NC-D particles 0.5mg/ml for 24 hours. Cells typically take up 20-30pg iron oxide.

Animal models. Two animal models have been set up using immunodeficient mice. 1) To develop lung cancer metastases: $2x10^6$ MDAMB231 breast cancer cells were injected intravenously (day 0). Tumors were imaged at day 28 and 38. To demonstrate homing of MSCs, $5x10^5$ DiI-MSCs were injected i.v. on day29 and excised on day30. 2) To assess the sensitivity of MR detection of iron-labeled cells: subcutaneous tumors were developed by co-injection of $2x10^6$ MDAMB231 cells with $5x10^5$ iron-labeled DiI-MSCs. Tumors were imaged at day29 and excised for histology.

MRI. Images were acquired on a 9.4T horizontal bore Varian VNMRS system using a 39mm RF coil. Lung *in vivo* images were acquired using a fast spin-echo sequence with cardiac and respiratory gating (TR~1s, EffTE=5ms, 100um res, 1mm slc). Subcuteneous tumor images were acquired *ex vivo* using the same sequence and similar parameters (TR=1.5s, EffTe=5ms, 100um res, 1mm slc).

Histology and staining. Lungs and subcutaneous tumors were dissected and stained with DAPI to fluorescently label cell nuclei. Subcuteneous tumors were stained with Prussian Blue to detect iron, by incubating at 10% v/v HCl and 5% w/v KCN for 20 mins. Images were acquired using standard and fluorescent microscopy and the samples were inspected for presence of tumor metastases, MSCs and iron.6

Results: Fig 1 displays lung metastases at day30 with MSCs homing to these tumor sites. Lung metastases could be visualized *in vivo* using MRI at day38, but not at day28 (Fig 2). Fig 3 displays the heating response of NC-D particles to application of an RF field. MSCs readily take up these particles (not shown). MRI was sufficiently sensitive to detect 1000 labeled MSCs injected into tumors (Fig 4).

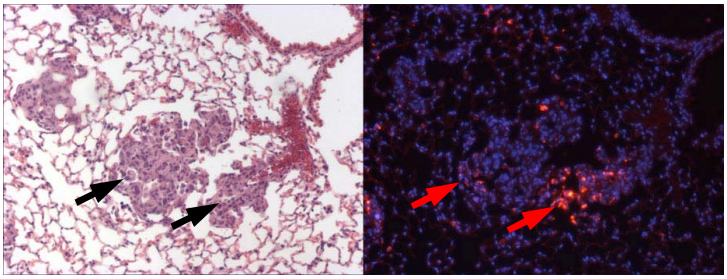

Figure 1: Lung metastases on day30 (black arrows) with DiI-MSCs (red)

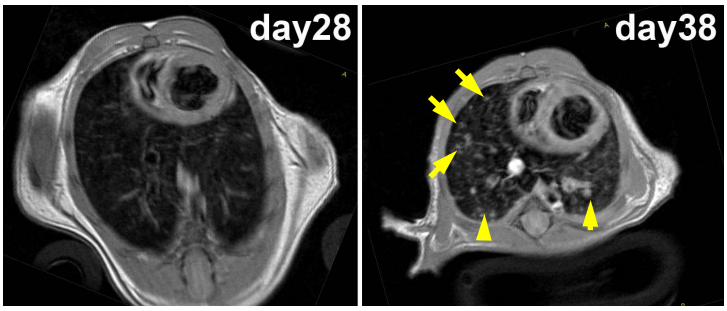

Figure 2: Lung metastases on MRI *in vivo*

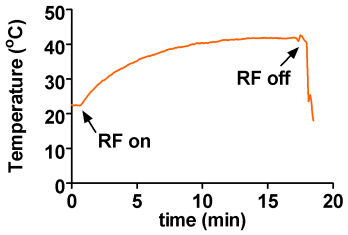

Figure 3: NC-D 200nm particle heating

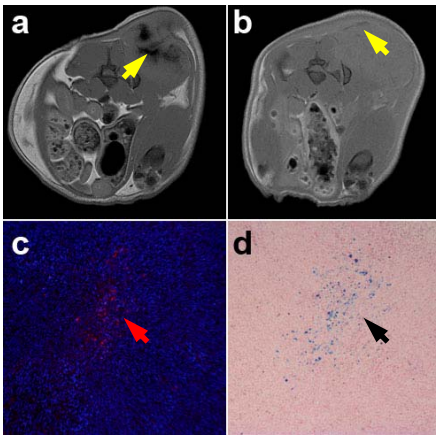

Figure 4: Subcutaneous tumors at day29 with a) 100,000 MSCs and b) 1,000 MSCs. DiI-MSCs (c) stain positively for iron (d).

Discussion:

There is a pressing need for adjuvant cancer treatments. In this preliminary study we have demonstrated the potential of the NC-D iron particles for targeted hyperthermia, the specific homing capacity of MSCs to sites of lung tumor metastases, and the possibility of loading MSCs with the NC-D particles for combined MR monitoring and specific delivery of a thermic load to sites of metastasis. In the future we plan to use this technology to cause tissue damage within tumors as an additional therapeutic approach to chemo- and radiotherapy.

References: 1. Giaccone. *Oncogene* 21, 6970-812 (2002). 2. Nakamizo et al. *Cancer Res* 65, 3307-18 (2005). 3. Studeny et al. *Cancer Res* 62, 3603-8(2002). 4. Pankhurst et al. *J Phys D* 36, R167-81 (2003). 5. Kallumadil et al. *J Magn Magn Mater* (2009, in press)

Acknowledgements: We are thankful for support from the BHF, the MRC, the UCL ICH CHRAT and the A.S. Onassis Public Benefit Foundation.